HIGH SCHOOL EDITION
Journal of Student Research

Volume 12 Issue 1 (2023)

Adjusting pH Levels in Respect to Crustacean Hatching Rates

Jessica Kerul[1] and Patricia Talarczyk[#]

[1]Mentor High School, Mentor, OH, USA
[#]Advisor

ABSTRACT

Currently, studies have been conducted focusing around aquatic species and how chemical properties within the water may impact their survival, such as the effect of pH levels on flounder eggs. In specific, the study discussed how acidic pH levels negatively affected their development and survival rate. However, there is limited research conducted pertaining to the connection between pH levels and crustaceans. This begs the question: how do differing pH levels in water affect the hatching rates of aquatic organisms, specifically crustaceans? Based on previous studies, if crustaceans are exposed to acidic pH levels, then the hatching rates will be negatively affected. In order to prove this, an experimental method was used with five samples with different pH levels and sea monkeys added to them. Sea monkeys, a hybrid breed of brine shrimp, were used in order to represent crustaceans in the experiment. The five samples were recorded hourly for times of initial hatching and the relative amount of eggs hatched. Concluding the experimental process the results showed that the basic pH levels had negative effects towards the hatching rates with no survival at 8.0 and very little survival at 7.5. Also, there was an increased rate of hatching for the acidic pH levels with the highest abundance of sea monkeys and the quickest to hatch at 6.0. Overall, the data concludes that the basic pH levels negatively affect the hatching rates of crustacean eggs, and the acidic pH levels had little effect on the hatching rates.

Introduction

According to Melissa Denchak from Natural Resources Defense Council, the ocean absorbs almost a quarter of all man-made carbon-based air pollution, also impacting pH levels throughout the water (Denchak 2018). Earth's bodies of water can easily be polluted or contaminated by natural sources and man-made sources, such as anything from mine sites to an increased abundance of metals within the water. All of these changes will impact the ecosystem, including by affecting the pH level within the water.

Bodies of water can be measured by how acidic or basic the water is by the pH scale. The pH scale measures zero to fourteen and is impacted by the concentration of hydrogen ions on a logarithmic scale, meaning that as the scale increases by one, the concentration of hydrogen ions decreases by ten (Huber and Blaha-Robison [date unknown]). In addition, zero to seven is considered to be acidic, with a higher concentration of hydrogen ions, and seven to fourteen is considered as basic, with a lower concentration of hydrogen ions. When a liquid or solution has a pH level of seven, it is considered to have a neutral pH, not acidic nor basic, which is the level of distilled water. A normal range for water sources is, roughly, six to eight (Drinking… 2019). In connection with large bodies of water, the ocean is measured at 8.2 pH level, on average, and freshwater is measured at around 7, on average. However, these conditions can easily change based on contaminants that can be brought into waterways. For instance, the pH level can decrease from mine sites, acidic soil, or runoff and the pH can increase from soap manufacturing, asphalt, and alkaline soils (pH 2018). There are many other factors that can impact the pH levels, including natural factors, however any factor can show consequences within an ecosystem.

The consequences begin with the smaller organisms, including crustaceans, which are mostly aquatic species that are a part of arthropods and only have an exoskeleton (Crustaceans). These shells are hard and symmetrical down the middle of the organism (Crustaceans). Some examples of crustaceans are shrimp, brine shrimp, barnacles, and crabs (Crustaceans). Due to this hard exoskeleton, the organisms require a large supply of carbonate ions in the water in order to survive and grow (Ocean Acidification). In other words, the carbonate is widely used by many species, especially crustaceans, because it allows the organisms to build their shells and skeletons. Also, crustaceans do not grow with their exoskeleton, meaning that they must shed their old shell once their new shell has formed beneath it (Crustaceans). Therefore, this process in crustaceans requires a constant supply of carbonate within the water to build the strong exoskeleton. On a different note, most crustaceans reproduce sexually and produce viable eggs, and others can produce fertilized eggs by solely the female (Crustaceans). By producing eggs, the eggs do not have the open circulatory system like the adult organisms do (Crustaceans) and must rely on the water flow in order to receive the oxygen and nutrients they need (Ocean Acidification). Therefore, the contents within the water are essential for these organisms' survival and can affect their development greatly if there are harmful changes.

In connection, these harmful changes include inconsistent pH levels of the water, which can be extremely harmful to the aquatic eggs. First off, most aquatic organisms thrive in pH levels of 7.8 to 8.2 and since crustaceans require high levels of carbonate in the water, it is important that the pH level stays constant, due to the carbonate levels being affected by changes in the pH level. For instance, acidic levels have reduced levels of carbonate in the water (Ocean Acidification). This process is because of the increased abundance of carbon dioxide in the water and the chemical reactions that occur with the carbon dioxide, forming hydrogen ions and bicarbonate, which cannot be used by the organisms (Influence of Osmolality). In contrast, when the pH levels increase, carbon dioxide levels decrease and there is an increase in carbonate levels. On another note, the Environmental Protection Agency (EPA) discusses how the low pH levels can lower growth, reproduction, and important bodily functions in organisms (pH). Similarly, the high pH levels can also show lower growth rates, lower reproduction rates, and damage organs (pH). Although changes in pH may be slight, the consequences are important for any aquatic species, especially crustaceans.

Within any major body of water, little changes to the ecosystem can be critical to the organisms and their supply of nutrients. However, there is limited amounts of information that can inform society about how crucial pH levels are to the ecosystem, especially with changes in pH. The pH levels may be caused by chemical changes or pollutants within the water, however, they impact organisms in many ways, including the development of aquatic eggs. The changes in pH levels can be from a variety of reasons and can shift the pH up or down. For instance, the EPA claims that some of the reasons for low pH levels from pollutants are from mine sites, contaminated runoff, and from metal or chemical pollution (pH). Another claim from the EPA is that some of the reasons for increased pH levels are from asphalt production, oil production, and soap manufacturing (pH). Once these pollutants have entered the body of water, all organisms are impacted, including crustacean eggs. Although aquatic crustacean eggs are so little, they impact the entire ecosystem greatly, especially due to the fact that they are food supply for the larger organisms (Conditions Necessary…).

Currently, there are a few studies conducted about how pH levels can impact organisms, including aquatic species and their eggs. For instance, Sambid Swain, et al from the Journal of Entomology and Zoology Studies discusses how ocean acidification, which is where the pH level of the ocean has been decreasing due to increased levels of carbon dioxide in the atmosphere, has been affecting the development of flounder eggs (Effect of Water pH on the Embryonic…). Also, the article suggests that the reason for this is because the eggs have a harder time maintaining homeostasis with the lower pH levels (Effect of Water pH on the Embryonic…). Similarly, a study by AA Faris and RJ Wooton from the National Center for Biotechnology Information discuss how a fish species, euryhaline teleost, Gasterosteus aculeatus, had a decreased survival rates with low pH levels (Effects of Water pH and Salinity…). In addition, they claimed that the eggs had a greater survival rate when they were exposed to higher concentrations of salinity in the water (Effects of Water pH and Salinity…). Lastly, the article "The Effects of pH on the Abundance of Phytoplankton for Mariculture," by Micah Briola, discusses how Phytoplankton, or shellfish, were used in an experiment to find out if pH levels affected their population and growth (The Effects of pH on the Abundance…). From the experiment, Briola found that although the data was not clear, the phytoplankton had the most success at the pH level of 7.8 (The Effects of pH on the Abundance…). These studies have shown that aquatic organisms thrive in neutral pH levels and are impacted greatly by changes in pH levels. However, the studies propose a gap in the research about how crustaceans, and their eggs, are impacted by pH levels, above and below neutral levels.

Therefore, with limited information about how differing pH levels impact the hatching rates in crustaceans, including the impacts of above neutral pH levels on aquatic life. Thus, in connection to pH levels affecting the hatching rates of aquatic eggs, such as crustaceans, it begs the question, how do differing pH levels in water affect the hatching rates of aquatic eggs, specifically crustaceans?

Method

It is evident that pH levels impact aquatic life due to many reasons, including chemical changes. Although the pH level specifically measures the concentration of hydrogen ions, the concentration of carbonate ions is what impacts the organisms the most. This leads to the question: How do differing pH levels in water affect the hatching rates of aquatic eggs, specifically crustaceans?

One animal that represents crustaceans are prehistoric sea monkeys. Sea monkeys are man-made organisms that are a hybrid breed of brine shrimp that are in an cryptobiosis stage (Harvey 2022). This stage is when organisms undergo a "state of suspended animation" until conditions improve, or in an anhydrobiotic state, and they hatch into living organisms, which is one reason that they are beneficial for research and easy to travel and manipulate (Schwarcz 2017). The creatures were originally food for fish until a scientist found a better use for them and experimented with ways to preserve the eggs and make them "come to life" with the right conditions, which creates the idea of instant life (Harvey 2022). In addition, these organisms are great examples of crustaceans since they only have an exoskeleton and they are a specific breed of brine shrimp, which organizes them as crustaceans (Crustaceans 2015). Although they are referred to as sea monkeys, they are not monkeys at all and they are considered tiny brine shrimp eggs with tails that resemble the tails of monkeys (Schwarcz 2017). On another note, the sea monkeys reproduce sexually or asexually (Harvey 2022) in the same way that crustaceans do (Crustaceans 2015).

Along with the sea monkey eggs, other materials were used in order to conduct the final results. These materials included purified water, five plastic containers, multiple pH level measuring sticks, white distilled vinegar, and baking soda. The plastic containers and sea monkeys eggs were supplied by Discovery Prehistoric Sea Creatures, at-home STEM Kits (Appendix B). The baking soda and distilled vinegar were used in order to manipulate the pH levels, in specific the baking soda was used to increase the pH, and the distilled vinegar was used to decrease the pH. According to the article, "Conditions Necessary for the Life of a Brine Shrimp," sodium bicarbonate, or similarly known as baking soda, can be used to increase the pH level of the water with brine shrimp (Conditions Necessary…[date unknown]. The author concluded that In addition, discusses how white vinegar is used to decrease the pH level in drinking water and is safe for organisms to consume (Drinking Water…2019). Lastly, the pH level measuring sticks were used to dip into the five water samples in order to test the pH levels after manipulations were made.

For the experimental process, it began with five plastic containers, labeled one through five, filled with 16 ounces of purified water. In sample one, ½ teaspoon of white vinegar was mixed into the water or until the sample reached a pH level of 5.5. In sample two, only ¼ teaspoon of white vinegar was mixed into the water or until the water reached a pH level of 6.0. In sample three, the water was not manipulated to any extent. In sample four, ¼ teaspoon was mixed into the water or until the pH level reached 7.5. Lastly, in sample five, ½ teaspoon was added and mixed into the water or until the pH level reached 8.0. In order to measure the pH levels in each sample, pH level strips were used (Appendix C).Immediately, the packets of sea monkey eggs were added to each of the containers and started the experiment, this time was recorded. During the experiment, the samples were checked for any sign of movement floating, similar to small tadpoles, in the water every hour and the data was recorded (Discovery Prehistoric…2011). According to the Discovery Prehistoric Sea Creatures, at-home Kits, any debri in the water will sink to the bottom and the eggs will float on the surface (Discovery Prehistoric…2011). Therefore, when the eggs hatched, they were visible with a black background behind each container and appeared to be small, white, moving dots (Ludemann 2021). Once there were any signs of movement within the water samples, the time was recorded for the specific sample as the crustaceans began the hatching phase. This continued until all of the samples began the hatching process. Finally, after five hours following the hatching process recorded time, the samples were checked for the relative amounts of sea monkeys hatched. This was recorded in comparison to each other based on the amount of organisms moving within the water. The final results were recorded in a Google Sheets labeled with each sample number and the pH level they corresponded with (Appendix A). This concluded the experimental process.

The experimental process involved a primary variable, the pH levels, that would influence the participants, the sea monkeys, in order to determine the hatching rates in comparison with each of the results. In addition, the process included secondary variables of how the pH levels impacted the amount of eggs hatched in respect to each other after the hatching processes began. On another note, the control variables included the amount of light exposed to the samples, the amount of water used per sample, the amount of eggs used, the temperature, and the volume of each container. These control variables limited the amount of confounding variables, which are variables not included in the experiment that may cause skewed results, due to having all five samples under the same conditions at all times.

Results

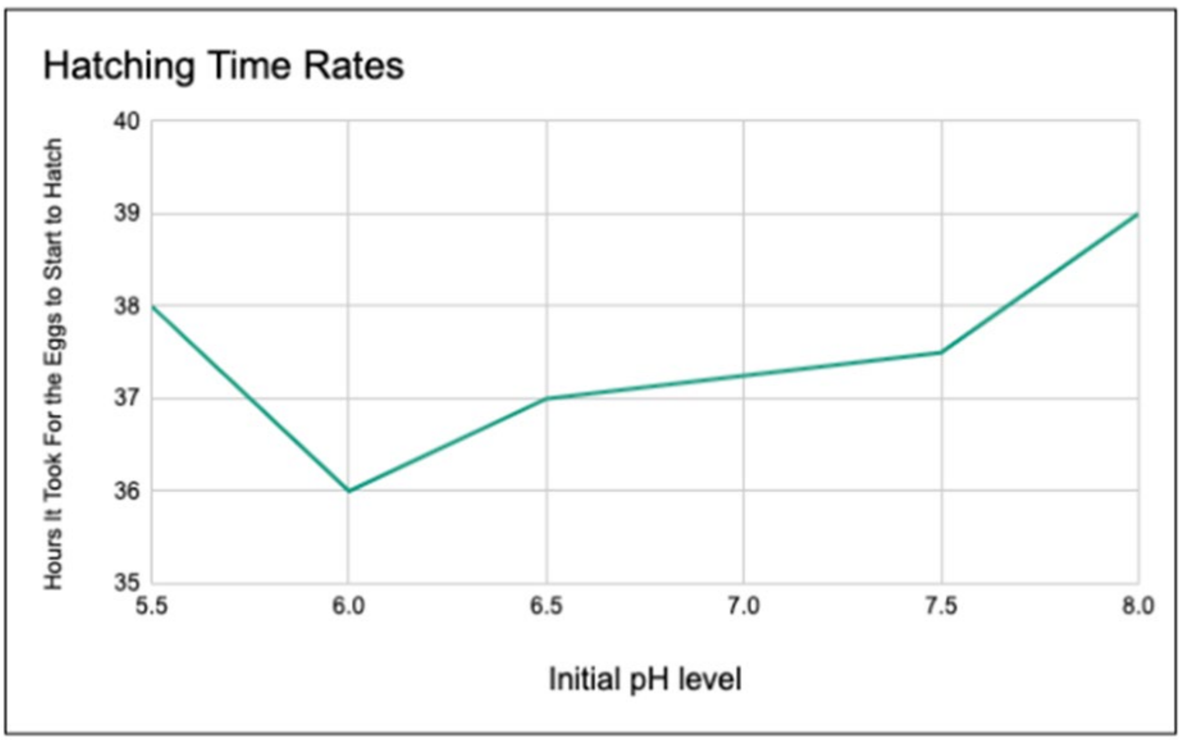

Figure 1. Time of Initial Hatching Versus pH Level

From the experimental method process, the samples were recorded based on the times the sea monkeys took to initially hatch and the overall abundance of hatched sea monkeys. For example, Figure 1 displays the initial hatching times with Sample 2, at a pH level of 6.0, was the first to start hatching at around 36 hours. Following, Sample 3, at a pH level of 6.5, hatched at 37 hours and then Sample 4, with a pH level of 7.5, at 37.5 hours. Lastly, Sample 1, with a pH level of 5.5, hatched around 38 hours and Sample 5, at a pH level of 8.0, hatched around 39 hours. Furthermore, the pH levels of 6.5 and 6.0 showed the most positive hatching rates with the quickest initial hatching times and the best relative abundance in comparison to all five samples. As for the relative abundance of hatched sea monkeys, Sample 1 had very little abundance of hatched sea monkeys and Sample 2 had the highest abundance of hatched sea monkeys. Also, Sample 3 had a small abundance of sea monkeys; Sample 4 had a very little abundance of sea monkeys; and lastly, Sample 5 had no abundance of sea monkeys hatched. The samples closer to neutral, or seven, had the shorter hatching times and the samples farther from neutral, such as samples 1 and 5, had longer hatching times. Thus, samples 1 and 5, with pH levels of 5.5 and 8.0, show the most negative impacts on the hatching rates with the longest hatching times and the least relative amounts of hatched eggs. In connection with crustaceans, the sea monkeys allow the data to represent what is present with the hatching rates of all crustaceans at the corresponding pH levels. Therefore, the basic pH levels negatively affect the hatching rates of crustaceans, and the acidic pH levels show positive effects on the hatching rates. In other words, if the pH levels drop below 7, crustaceans will be able to hatch and survive; however, if the pH level rises about 7, then crustaceans will not hatch properly or even survive, especially with pH levels above 8.0. Evidently, there is a new understanding that addresses how differing pH levels greatly impact the crustacean eggs, along with all aquatic eggs, in a variety of ways, including the basic pH levels decreasing the hatching rates and the acidic levels having little effect on the hatching rates.

Conclusion

Nonetheless, the impact that pH levels have on crustaceans and their hatching rates may allow more conclusions to be made involving aquatic ecosystems. The pH levels are constantly changing within bodies of water and the results of my data can show how some of these levels may affect the organisms. First of all, these pH levels are influenced by many factors, such as burning fossil fuels, acidic soil runoff, and oil production pollution, which were discussed in the article "pH." With that being said, my data shows how basic pH levels can negatively affect the hatching rates of crustaceans, which can correlate to other organisms within the ecosystem. Specifically, the organisms that would correlate the most would be any species that has an exoskeleton or a vertebrae, which requires the carbonate ions from the pH levels. In other words, any organism that contains a carbon-based structure would relate closely to crustaceans and to the data found in the experiment. Therefore, the data concludes that since the basic pH levels had more negative effects on the hatching rates of crustaceans, these species will have similar hatching effects, which could propose a danger for the species. Although not all aquatic organisms have a carbon-based structure, all organisms require nutrients from the water and pH levels are a great indicator of the nutrient concentration, especially the hydrogen and carbon dioxide concentration. The carbon dioxide concentration can be determined by the pH because when carbon dioxide and water react, they form hydrogen ions, which is used to measure pH (Sensorex 2020). Besides the carbon dioxide, the pH level can be impacted by the nutrients within the water and can impact species in negative ways. All in all, my results suggest that the basic pH levels would be more harmful towards the organisms than the acidic pH levels, which not only impacts crustaceans, but it can impact other organisms, as well.

The impact of pH levels on aquatic organisms stresses the importance of data collection and information on these species. For instance, the world is constantly changing and so are the pH levels in a body of water. The constantly changing pH levels then affect organisms in different ways and differing severities. The smaller organisms may be affected more from the levels than the larger organisms, which change the dynamic of an aquatic ecosystem. Although the larger organisms may not be affected as much, they rely on the negligible organisms for food and nutrients. In other words, the pH levels may affect the smaller organisms directly, but they greatly impact the larger organisms, as well. The importance of gathering information on how smaller organisms are affected by the pH levels can benefit the larger organisms and their food sources immensely. In connection to humans and society, the larger organisms may be used for food and nutrients, like different types of fish. There are countless aquatic species that are found on restaurant menus or used for meals across the world as large sources of protein. All of these species may be directly or indirectly affected by the changing pH levels found in bodies of water. Thus, gathering information on pH levels affecting aquatic organisms can be beneficial to predators in the ecosystem and for maintaining access to large sources of protein.

With differing pH levels impacting the hatching rates of crustaceans, this information is able to connect with previous studies in various ways. For instance, the negative effects of the basic pH levels on the hatching rates contradicts the ideas presented by Swain, et al, with discussing how the acidic pH levels harm the development of flounder eggs. Specifically, Swain argues that the acidic levels are more harmful due to their effect on the flounder's homeostasis, and my data shows how the acidic pH levels had a neutral or positive effect on the hatching rates of crustaceans. This difference between studies allows the scholarly conversation to include various studies with different organisms that can replicate how an ecosystem may be impacted by the differing pH levels. Similarly, my new understanding refutes the ideas claimed by Faris and Wooton about how the acidic pH levels negatively affected the survival rate of larvae, when my results contradicted that the acidic pH levels showed positive hatching rates for crustaceans. Apart from the use of different organisms, these sources and my data, covering the impacts that pH levels had on crustaceans, contradict on how acidic and basic pH levels impact the survival rate or development of aquatic organisms. As a result, these differences amplify the importance of this study as not all organisms react the same to the pH levels.

Overall, the data shows that the basic pH levels are harmful to crustaceans, and all aquatic eggs, and acidic pH levels are not harmful. Nevertheless, the data may not fully truly represent how the changes in pH will affect the hatching rates of all crustaceans. First of all, this may be because the sea monkeys are only a hybrid breed of brine shrimp, meaning that they are manipulated by scientists and can have different hatching processes than what traditional brine shrimp have. In other words, the hatching rates may not be the same or react the exact same for the crustaceans in comparison to the sea monkeys with the differing pH levels from the experiment. Although sea monkeys are considered to be brine shrimp, if this was not an issue, the data would be able to ensure that the results represent how differing pH levels would affect brine shrimp and crustaceans. One way that may have been able to eliminate this issue was by using a crustacean, such as brine shrimp, as my experimental sample, however I did not have access to these organisms due to a lack of accessibility. On another note, brine shrimp are only one species within all crustaceans, which limits how the data can represent crustaceans, as a whole. This would allow the data to correlate to all crustaceans, however, this issue is also caused due to the lack of access to true crustaceans. These limitations are important because they affect the strength of my new understanding of how differing pH levels affect the hatching rates of aquatic eggs, specifically crustaceans, by not fully representing the crustacean population.

Ultimately, the limitations of my data propose areas of research that can be conducted to further support my new understanding of basic pH levels negatively impacting the hatching rates of aquatic eggs, specifically crustaceans. Since, the sea monkeys used in the experiment are only a hybrid breed of brine shrimp, this opens future questions about how specific crustaceans and if they are influenced by the pH levels the same way that my data suggests. This future research would continue to support my new understanding focusing on crustaceans and how they can be in possible danger to do changes in the water. Furthermore, it will add to the overall scholarly conversation surrounding the development of aquatic species. Equally important, another area of research that can be conducted is the idea of testing the salt concentration with the effect it has on crustaceans. According to Faris and Wooton, with higher salinity levels in the water, the survival rate of the larvae increased. This study presents the possible impact of salt concentrations on crustaceans and their hatching rates (Faris and Wooton 1987). With this information, connections can be drawn between the effects of salinity and pH levels on crustaceans and to each other. In other words, the research may answer if there is a correlation between pH levels and salt concentration in the water and if this connection positively or negatively affects the aquatic organisms. Not only do my results show there is a correlation between different pH levels and the hatching rates of crustaceans, there are many future areas of research to build off of my data and to strengthen my new understanding.

In conclusion, differing pH levels greatly influence the hatching rates of aquatic eggs, especially crustaceans, in various ways. For instance, the basic pH levels, or levels above seven, negatively affect the hatching rates and the acidic pH levels, or levels below 7, have little to no effect on the hatching rates of the crustaceans. The data came from the experimental process of five different samples of sea monkeys with different pH levels in each container, which were recorded hourly for the initial hatching times and the overall relative abundance of hatched eggs in each sample. In particular, this experiment is important because pH levels are impacted by multiple factors, including burning fossil fuels, oil spills, and acidic soil runoff, which influence the nutrients needed by the organisms, such as carbonate ions. These carbonate ions allow aquatic species, especially crustaceans, to build a hard exoskeleton around the body's organs in order to survive. In addition, the most impacted organisms can also impact an entire ecosystem and food sources. Thus, as for all aquatic organisms, data needs to be collected surrounding how pH levels affect each type of organism before it becomes too late.

Acknowledgments

I would like to thank my advisor for the valuable insight provided to me on this topic.

References

Briola, M. (2019, October 10). *The effects of ph on the abundance of phytoplankton for Mariculture*. ScienceBuzz. Retrieved December 14, 2021, from https://www.sciencebuzz.com/the-effects-of-ph-on-the-abundance-of-phytoplankton-for-mariculture/

Conditions Necessary For the Life of a Brine Shrimp. Science Projects. Retrieved February 14, 2022, from https://www.scienceprojects.org/conditions-necessary-for-the-life-of-a-brine-shrimp/

Crustaceans. Marine Education Society of Australasia. (2015). Retrieved February 14, 2022, from http://www.mesa.edu.au/crustaceans/

Denchak M. 2018 Jan 22. Ocean Pollution: The Dirty Facts. NRDC. https://www.nrdc.org/stories/ocean-pollution-dirty-facts.

Drinking-Water. (2019, August 23). Drinking water treatment – PH adjustment. Drinking Water and Human Health. Retrieved October 14, 2021, from https://drinking-water.extension.org/drinking-water-treatment-ph-adjustment/.

Environmental Protection Agency. *The Effects: Environment*. EPA. Retrieved February 28, 2022, from https://www.epa.gov/nutrientpollution/effects-environment#:~:text=Algal%20blooms%20can%20reduce%20the,recreation%2C%20businesses%20and%20property%20values

Faris, A. A., & Wootton, R. J. (1987). *Effect of water ph and salinity on the survival of eggs and larvae of the euryhaline teleost, gasterosteus aculeatus L*. Environmental pollution (Barking, Essex : 1987). Retrieved February 14, 2022, from https://pubmed.ncbi.nlm.nih.gov/15092698/#:~:text=The%20survival%20of%20eggs%20from,an%20effect%20on%20egg%20survival.

Goettsche Partners. (2011). *Discovery Prehistoric Sea Creatures, at-Home STEM Kits for Kids, Sea Creature Aquarium, Grow Your Own Creatures, Hatching Animals, Kids Science Activities*. Amazon. Retrieved February 28, 2022, from https://www.amazon.com/gp/product/B08GYH7DZ1/ref=ask_ql_qh_dp_hza

Hadly, S. (2021, January 12). Ocean acidification linked to plastic pollution- study. Earth.Org - Past | Present | Future. Retrieved October 14, 2021, from https://earth.org/ocean-acidification-linked-to-plastic-pollution/.

Harvey, A. (2022, February 4). *What are sea-monkeys?* LiveScience. Retrieved February 14, 2022, from https://www.livescience.com/33907-sea-monkeys.html

Huber, M., & Blaha-Robinson, K. *Algae culture and ph*. Algae Research Supply. Retrieved December 14, 2021, from https://algaeresearchsupply.com/pages/algae-culture-and-ph#:~:text=For%20example%2C%20marine%20algae%20strains,affect%20how%20much%20is%20available

Kim, K.-H., Moon, H.-N., Noh, Y.-H., & Yeo, I.-K. (2020, March). *Influence of osmolality and acidity on fertilized eggs and larvae of olive flounder (paralichthys olivaceus)*. Development & reproduction. Retrieved February 14, 2022, from https://www.ncbi.nlm.nih.gov/pmc/articles/PMC7201059/

Ludemann, D. (2021, November 1). *How to care for Sea Monkeys*. 13 Steps (with Pictures) - wikiHow Pet. Retrieved February 14, 2022, from https://www.wikihow.pet/Care-for-Sea-Monkeys#:~:text=Put%20the%20sea%20monkeys%20in,wait%20for%20them%20to%20hatch.&text=The%20sea%20monkeys%20will%20appear,once%20to%20twice%20a%20day.

Schwarcz, J. (2017, April 3). *The right chemistry: Monkeying around with 'sea-monkeys'*. McGill Office for Science and Society. Retrieved February 14, 2022, from https://www.mcgill.ca/oss/article/general-science/right-chemistry-monkeying-around-sea-monkeys

Swain, S., Banerjee Sawant, P., Kumar Chadha, N., Sundaray, J. K., & Prakash, C. (2020). *Effect of Water pH on the Embryonic Development of Discus, Symphysodon Aequifasciatus, Pellegrin, 1904*. Journal of Entomology and Zoology Studies. Retrieved February 14, 2022, from https://www.entomoljournal.com/archives/2020/vol8issue3/PartZ/8-3-193-993.pdf

The Ocean Portal Team. (2019, June 20). *Ocean Acidification*. Smithsonian Ocean. Retrieved February 14, 2022, from https://ocean.si.edu/ocean-life/invertebrates/ocean-acidification

Understanding the ocean acidification equation and its biological impact. Sensorex. (2022, February 14). Retrieved April 25, 2022, from https://sensorex.com/2020/05/19/understanding-the-ocean-acidification-equation-and-its-biological-impact/

US EPA,ORD. 2018 Nov 6. pH | US EPA. US EPA. https://www.epa.gov/caddis-vol2/caddis-volume-2-sources-stressors-responses-ph

Wildman R, Benner B, Held D, Schauberger C. 1974. Influence of Blue-Green Algae on the pH and Buffer Capacity of Culture Media. Proceedings of the Iowa Academy of Science Proceedings of the Iowa Academy of Science. 81(4):192–196. [accessed 2021 Oct 18]. https://scholarworks.uni.edu/cgi/viewcontent.cgi?article=1552&context=pias.